BioChemistry

SCREENING OF A BOVINE cDNA LIBRARY FOR eIF-5A AND DEOXYHYPUSINE SYNTHASE CLONES. <u>Valerie C. Sershon</u>, Jason C. McDonald, Austin L. Barclay, Waseem M. Kiyani, Jenq-Kuen Huang*, and Lisa Wen* Department of Chemistry, Western Illinois University, Macomb, IL 61455, vc-sershon@wiu.edu

Hypusine (4-amino-2 (R)-hydroxylbutyl) lysine) synthesis in a mature eukaryotic initiation factor-5A (eIF-5A) is a unique two-step posttranslational modification involving two enzymes, deoxyhypusine synthase (DS) and deoxyhypusine hydroxylase (DH). Inhibitors of either the DS or DH have been shown to exert antiproliferative and antiretroviral effects. Since DH has not yet been purified to homogeneity, our long-term objective is to purify and characterize the enzyme. A detailed knowledge of structure-function relationship of this enzyme will help in designing specific inhibitors. Bovine testis is an excellent source of DH. To develop an assay for bovine DH, both bovine DS and eIF-5A precursor proteins are essential.

We report here screening of a bovine cDNA library (a generous gift from Dr. John A. Glomset of University of Seattle) using human eIF-5A and human DS cDNAs as probes. A total of 2.5 x10⁵ clones from a ZAP ExpressTM/*EcoR I/Xho I* bovine cDNA library was screened with the mixed human probes. A total of 250 clones were hybridized to the probes. Thirty-three clones were subjected to a secondary screening and 30 clones were isolated. These clones will be characterized until full-length cDNAs for eIF-5A and DS are obtained. Each cDNA will be subcloned into an expression vector for protein expression.

This work is supported in part by grants from University Research Council, Western Illinois University, USDA CSREES 99-34401-8312, and NIH, 1R15 GM60266-01A1.